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# The comparative value of various germicides for use in cane sugar factories

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# THE COMPARATIVE VALUE OF VARIOUS GERMICIDES FOR USE IN CANE SUGAR FACTORIES.

## INTRODUCTION.

It is quite probable that the practice of using some form of germicide for preventing the growth of microorganisms in sugar factories existed at an early stage of the development of the sugar industry. This practice today, just as it was at a much earlier period, is almost entirely lacking in uniformity, and may be regarded as a purely empirical process. The use of formaldehyde as a preservative of juices in cases of emergency is, on the contrary, based upon the results of special investigation and is a thoroughly efficient procedure. But when it becomes necessary to outline a systematic campaign for cleaning sugar factories no data is obtainable upon the kind and amount of germicide that would prove most efficient under these conditions. So it frequently happens that whether formaldehyde or milk of lime, or some other germicide is used for this purpose, depends more upon the convenience of their respective use than their relative efficiency under the given conditions. Such haphazard methods could scarcely be expected to prove efficient, for should they happen to be effective in any instance it would probably be at an excessive cost.

There has recently been an awakening of interest among sugar manufacturers, in the importance of maintaining cleanlier conditions in the sugar factory. The importance of manufacturing raw sugars that can be kept in storage without deterioration has been fully impressed upon the manufacturers by the losses which they have frequently incurred from this source. That sugar deterioration is in a large measure due to the lack of care in maintaining the proper conditions in the factory is now so fully appreciated that few manufacturers are indifferent towards proposals for correcting these conditions. A question perhaps unheard of in the sugar industry ten years ago, which is now frequently asked, is: "What is the most economical and efficient germicide for sugar factory use?" To give this question a definite and authoritative answer has naturally necessitated a special investigation of the efficiency of germicides under sugar factory conditions. The reputed efficiency of most germicides has been derived from investigations of their action upon pathogenic species of bacteria, under conditions pertaining to pathology. Germicides vary in their action, not only with the species upon which they are tested, but also with the composition of the substance with which they must be brought in contact to perform their work. It is obvious therefore that we could not accept unreservedly the reputed values of these substances, should we attempt to apply them to sugar house conditions. The present investigation, the results of which are given in the following pages, is an attempt to standardize the present use of germicides in connection with the cleaning of sugar factories.

The thanks of the author is due Mr. Taggart, whose kind assistance, not only in the chemical analyses required in the investigation, but also by many helpful suggestions, greatly facilitated the progress of the work. To Mr. Inami, who, in the capacity of assistant during the latter stages of the investigation, rendered very faithful service, the writer also wishes to express his thanks. For the contribution by the Perth Amboy Co., of New York,, through their local agent, Mr. Bodenbender, of the formaldehyde used in the investigation, due thanks are here given.

# Chapter I.

## PLAN OF THE INVESTIGATION.

### SELECTION OF GERMICIDES.

For a germicide to be suitable for use in sugar factories it must conform to the following standards: (1) It must be cheap. (2) It must be easily obtainable. (3) It must be practically nontoxic to the human system, or if slightly toxic it must admit of being easily and completely removed from the surroundings in which it is used, so that it may be employed without danger. (4) It must possess a relatively high germicidal action. (5) Its germicidal action must be prompt as well as effective. With the exception probably of the last of these conditions their fulfillment by a germicide will appear obviously essential for sugar house use. Upon reflection it will appear equally essential that the germicide perform its work within a comparatively short period of time. In making a selection of germicides for sugar house use we are necessarily confined within a rather narrow range. The restrictions are greater than in the field of pathology, on account of the larger amounts of substance to be used, the greater complexity of conditions, and the much lower limit of price, beyond which their use would prove prohibitive.

The following germicides were selected for the investigation: formaldehyde, milk of lime, chloride of lime, sodium fluoride, ammonium fluoride, sodium bisulphite, and calcium bisulphite. Their selection, as well as the proportions in which they were used, was based upon the manner in which they have been employed either in the sugar industry or in other somewhat closely allied fields of work. The germicides were employed in the following strengths:

| | |
|---|---|
| Formaldehyde .................. | 0.5% |
| Chloride of lime.................. | 2.0% |
| Ammonium fluoride ............. | 5.0% |
| Sodium fluoride ................ | 5.0% |
| Milk of lime..................... | 5.0% |
| Bisulphite of lime............... | 5.0% |
| Bilsulphite of soda.............. | 5.0% |

Some of the uses of these germicides in the sugar industry, and in other closely related industries, are given as follows:

*Formaldehyde.*

Jorgensen ( [1] ) recommends the use of formaldehyde in 0.5 per cent strength, for use in breweries and distilleries, for the cleaning of vats. Its use in the beet sugar industry for the prevention of fermentation in the diffusion batteries has been recommended by O. Friedrichs ( [2] ), who secured a patent for its use in this connection. He recommends its use in the proportion of 2½ to 5 grams to 100 kilos of beets. A similar use for formaldehyde has been recommended by Schulz ( [3] ), F. Strohmer ( [4] ) and M. Gonnermann ( [5] ) and others.

(1) Microorganisms and Fermentation, p. 32.
(2) Lafar Technische Mykologie, p. 460.
(3) Deutsche Zucker Industrie, 1903. Bd. 28 S. 2017.
(4) Oestereich Ungarische Zeitschrift, 1905. Bd. 34 S. 685.
(5) Deutsche Zuckerindustrie, 1905. Bd. 30, 145-185.

Kamerling ( [1] ) recommended the use of very dilute solutions of formaldehyde in treating raw sugars to prevent their deterioration. The principal use of formaldehyde in the cane sugar industry at the present time is for the preservation of juices or syrups in emergencies, and for the preservation of laboratory samples. For the preservation of clarified juice Spencer ( [2] ) recommends the use of formaldehyde in the proportion of 3 cc. of formalin to a cubic foot of juice. In connection with the preservation of laboratory samples with formaldehyde, it is interesting to note the work of Norris ( [3] ) on the changes in the polarization of sugar solutions due to the addition of formaldehyde. Simpson ( [4] ) recommends the use of formaldehyde on all mill juice to prevent fermentation in the factory. He recommends its use in the proportion of one part of formaldehyde to 20,000 parts of juice.

*Chloride of Lime.*

This substance is recommended for disinfecting filter bags in breweries by H. Will ( [5] ), who advocates its use in the proportion of from 3 to 3½ kilos of chloride of lime per hectoliter of

(1) Archief voor de Java Suikerindustrie, 1900. Bd. 8 S. 527.
(2) Handbook of Cane Sugar Manufacturers.
(3) Use of Formaldehyde in Sugar Mills, 1908.
(4) Bulletin de l'Association des Chimistes de Sucrerie et Dist., Vol. 25.
(5) Desinfektion und Desinfektionmittel im Brauereibetrieb.

water. It is now extensively employed in the sugar industry for disinfecting bag filters, for which purpose it is generally employed in the strength of from one to two per cent.

*Ammonium Fluoride.*

Hydrofluoric acid and certain of its salts have a very strong germicidal action. The acid and its ammonium salt are used extensively in molasses distilleries for the prevention of the contamination of yeast with bacteria. Effront (1), who first introduced this use for fluorides, succeeded in acclimatizing yeast to as high a concentration of the acid as 36 grams per 100 liters. The ammonium salt which now is chiefly used for the purpose is employed in the proportion of 0.1 gram per liter. The use of ammonium fluoride for cleaning vats in distilleries and breweries is recommended by Jorgenson (2), who prescribes a 5% solution for this purpose. H. Winter (3) recommends the use of this substances in beet sugar factories as an excellent means of combatting the development of *Leuconostoc mesenteroides.* Saillard (4) recommends its use for preventing inversion of sucrose in the diffusion of batteries of beet sugar factories.

*Sodium Fluoride.*

Harloff (5) recommends the use of this substance in 1 per cent strength as a sugar factory germicide. He particularly advocates its use for cleaning the rolls of cane mills, as well as for the general germicidal needs to which milk of lime is at present put.

*Milk of Lime.*

Fresh milk of lime is recommended by Jorgensen (6) as a disinfectant for the walls and ceilings of breweries and distilleries. In the cane sugar industry it is extensively employed for cleaning the rolls of cane mills, and for all other germicidal purposes in connection with the cleaning of the factories. It has

(1) Bulletin de l'Association des Chimistes de Sucrerie et Dist. Vol. 25.
(2) Microorganisms and Fermentation, p. 32.
(3) Archief voor de Java Suikerindustrie, 1893.
(4) Circulaire hebdom du Syndikat des fabr. de sucre, 1900. S. 610.
(5) Plantation White Sugar Manufacture, p. 23.
(6) Loc. cit.

been shown by Winter [1], however, that milk of lime is particularly ineffective in preventing the development of *Leuconostoc mesenteroides.* The explanation of its ineffectiveness in this connection is very probably due to the fact that this particular species of bacteria is favored by a neutral or slightly alkaline reaction, and hence the use of lime is apt to produce the conditions favorable for its growth.

*Sodium and Calcium Bisulphite.*

Both of these germicides have been recommended by Jorgensen [2] for use in breweries and distilleries. Saillard [3] successfully employed both of these substances for preventing the development of microorganisms in the diffusion batteries in beet factories. L. Lachaux [4] very strongly recommends the use of calcium bisulphite as a germicide for beet sugar factories.

(1) Loc. cit.
(2) Loc. cit.
(3) Loc. cit.
(4) Bulletin de l'Association des Chimistes de Sucr. et Dist., 1893. Bd. 10 S. 501.

## METHOD OF TESTING GERMICIDAL ACTION.

The various methods for testing the efficiency of germicides, including the original one used by Koch [1] on anthrax spores, and the various modifications that other investigators have made of this method, are not strictly applicable to the present investigation. These methods consist in exposing a test organism, either attached to silk threads, as in the original method used by Koch, or in the form of a fresh culture in nutrient solution, to the action of the germicide to be tested. At frequent intervals of time after the test organism is brought in contact with the germicide, transfers are made to a suitable culture medium, and the time required for the germicide to prevent the growth of the species is taken as the criterion of the germicidal value of the substance. In an investigation of the value of a germicide for sugar factory use, we are not exclusively concerned with its action upon any one species of microorganisms, but also, and chiefly, with its power to destroy the various species that usually occur in the products of the factory. It is necessary therefore to test the germicide upon these species collectively, in

(1) Mitt Kais. Ges. Amt., 1881. Bd. 1 S. 33.

the product where they occur naturally, to determine the relative efficiency of germicides when used in sugar factories.

The method selected for testing the action of the various germicides was as follows: Stock solutions of the substances were freshly prepared for every experiment. The strength of these solutions were such as to contain the desired amount of germicide in twenty ccs. For example, the stock formaldehyde solution which was to be used in 0.5% strength was made of 2.5% strength. The solution that was to be used in 5% strength was made up in 25% strength, so that in both cases the amount to be used was contained in 20 cc of the stock solution. In making the experiments 20 cc portions of the germicide solutions were introduced into 100 cc volumetric flasks. The flasks were then filled to the mark with the substance upon which the germicides were to be tested. A few minutes were allowed to elapse between the filling of the flasks, in order to allow sufficient time for the transfers of the substance from each flask immediately at the expiration of the desired period of exposure. The control flask received 20 ccs of sterile water and was filled to the mark, as in the other cases. The comparison with the control could then be made on an equal basis, since the amount of dilution was equalized in both cases. At the end of the period of exposure, transfers were made with sterile pipettes to flasks containing sterile water. From these, transfers were again made to sterile petri dishes, upon which sterile raw juice agar was poured, after which the plates were incubated for three days at a temperature of 35°C. During the time that the substance was exposed to the germicide an opportunity was given it to have been thoroughly mixed by frequently shaking the flasks. The dilution flasks were always thoroughly shaken before transfers were made from them. Six plates were made from every flask, and an average taken of the colony development upon them. Control plates were made in every case to determine whether plates and media were sterile.

The strengths of the stock solutions were based upon the composition of the substances and not upon their active principle. In the case of the formaldehyde solution, which was prepared from formalin, the strength is given in terms of formaldehyde, and not on the basis of the 40% commercial article. On the other hand, the strength of the chloride of lime solution is expressed in terms of the entire substance, and not on the available chlorine, which was approximately 35%.

## Chapter II.

### EXPERIMENTS WITH FRESH CANE JUICES.

The following table shows the results obtained with the use of the various germicides upon cane juice in the manner described in the previous chapter. The period of exposure in this experiment was twenty minutes, and the temperature at which the germicidal action took place was that of the room, which was 25°C. The results reported in this table are so entirely representative of a large number obtained from similar experiments that it was deemed unnecessary to report other results. The figures representing the efficiency of the germicides and their formaldehyde coefficient were derived in the following manner: The efficiency of germicidal action can best be expressed by dividing the number of microorganisms killed by the number in the original solution. In order to get the comparative efficiency of several germicides, the strength in which they are employed must be taken into account. Any arbitrary method of comparison, therefore, which takes into consideration the total germicidal action of a substance, and the amount required to perform it, will suffice for the needs of the present investigation. The following method was adopted: For every one per cent of microorganisms that survived an exposure to a one per cent solution of the germicide, ten was deducted from one hundred. Expressed as a formula, this becomes

$$E = 100 - \left( \frac{\frac{10}{1}}{S} \times \frac{L}{1} \right)$$

E = Efficiency.
S = Per cent strength of disinfectant.
L = Per cent microorganisms surviving the treatment.

It becomes apparent from this formula that E is increased or diminished according to the strength of the substance required for complete germicidal action. It is increased in proportion to which applications of less than one per cent strength are capable of destroying in the substance treated, all of the microorganisms in excess of one per cent. Conversely, it is decreased in proportion to which strengths in excess of one per cent are required to produce the same results. Under other circumstances the expression of germicidal efficiency on this basis would not be entirely correct. In pathology, for example, a survival of one per cent of the bacteria in a substance disinfected might give the disinfectant an efficiency of zero, since the surviving species might render the substance treated no less dangerous as a source of infection. In this investigation, however, we are more concerned with a rapid and extensive diminution of microorganisms, rather than with a complete sterilization process. For example, a one-half of one per cent solution of formaldehyde might decrease the microorganisms in a cane juice to one per cent of the original, in a fifteen minute exposure, while to destroy the remaining one per cent might require a several hours' exposure to a five per cent solution. It is obvious, therefore, that in such cases a complete sterilization would not justify the cost of the process.

Since formaldehyde is by far the most powerful of the germicides that we are testing, it is taken as the standard with which the others are to be compared. Its value is stated as 1, and the value of the others in terms of it, and their formaldehyde coefficient, is derived by the following formula:

$$\text{F. C.} = \frac{\text{L} \times \text{S F}}{\text{L} \times \text{Sx}}$$

F. C. = Formaldehyde coefficient.
L. = Per cent microorganisms surviving treatment.
S. F. = Per cent strength of formaldehyde.
Sx = Per cent strength of germicide to be tested.

## Table I.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON FRESH CANE JUICES.

| Germicide | % Strength | Dilution of Plate | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coeficient |
|---|---|---|---|---|---|
| Control | ... | 1:1 mil. | 13,000 | .... | .... |
| Formaldehyde | 0.5 | 1:1000 | 30 | 98.85 | 1. |
| Sodium Bisulphite | 5.0 | 1:1000 | 152 | 42.0 | .0198 |
| Chloride of Lime | 2.0 | 1:1000 | 20 | 96.94 | 0.375 |
| Milk of Lime | 5.0 | 1:1000 | 36 | 86.2 | .0833 |
| Sodium Fluoride | 5.0 | 1:1000 | 104 | 60.0 | .0287 |
| Ammonium Fluoride | 5.0 | 1:1000 | 25 | 90.4 | 0.119 |

Period of Exposure 20′.
Period of Incubation 72 hrs.

From the above table it will be noted that formaldehyde is, as was to be expected, far more efficient as a germicide than any of the others. Its germicidal action in .5 per cent strength is greater than the others in 5 per cent strength, with the exception of chloride of lime and ammonium fluoride. Milk of lime seems superior to both sodium bisulphite and sodium fluoride. The germicidal rank of the substances in the above experiment is as follows:

Formaldehyde.
Chloride of lime.
Ammonium fluoride.
Milk of lime.
Sodium fluoride.
Sodium bisulphite.

### COMPARISON OF GERMICIDAL EFFICIENCY AT VARIOUS TEMPERATURES.

It is a well-known fact that the efficiency of germicides increases with elevations of the temperature at which they are allowed to act. That this accelerated action produced by elevated temperature varies with the germicidal substance is generally understood. In order to test the influence of elevated temperatures upon the various germicides, and to determine the range of temperature in which the acceleration of germicidal action was greatest, the following experiments were conducted. The methods employed in these experiments were the same as those previously described, except for the fact that the flasks in which the juice was treated were kept at a definite temperature during the period of exposure. After introducing the germicide into the flask, the juice was brought to the desired temperature by heating in a water bath and the flask filled to the mark with it, as before. After filling to the mark, the flasks were again placed in a water bath and kept at the desired temperature during the period of exposure.

TABLE II.

SHOWING GERMICIDAL ACTION OF FORMALDEHYDE UPON FRESH CANE JUICE AT VARIOUS TEMPERATURES.

| Strength of Germicide | Temp. | Dilution of Plates | Average No. of Colonies | (+ 000) No. of Micro. per c. c. | Efficiency |
|---|---|---|---|---|---|
| Formaldehyde 0.5%............ | 25° C | 1:1000 | 51 | 51 | 97.5 |
| Control........................ | 25° C | 1:1 mil. | 10 | 10,000 | ... |
| Formaldehyde 0.5%............ | 35° C | 1:1000 | 32 | 32 | 98.25 |
| Control........................ | 35° C | 1:1 mil. | 9 | 9,000 | ... |
| Formaldehyde 0.5%............ | 45° C | 1:1000 | 18 | 18 | 98.88 |
| Control........................ | 45° C | 1:1 mil. | 8 | 8,000 | ... |
| Formaldehyde 0.5%............ | 55° C | 1:1000 | 5 | 5 | 99.65 |
| Control........................ | 55° C | 1:100,000 | 70 | 7,000 | ... |

Period of Exposure 15'.
Period of Incubation 72 hrs.

It will be noted from the table that the acceleration of the germicidal action of formaldehyde by elevated temperatures is very gradual. A rise of thirty degrees centigrade resulted in only 2.1% increase in germicidal efficiency, which is .7 of one per cent for each ten degrees' increase in temperature. In each case the comparison is made with a control exposed to the same temperature, in order that the detrimental action of the temperature might not be included in the germicidal action. The decrease in the number of microorganisms in the control juice resulting from the rise in temperature from 25°C. to 45°C. can scarcely be attributed solely to the effect of the temperature. It is probable that it is due to a large extent to the expansion of the solution, which would result in fewer numbers of microorganisms being contained in one cc than when measured at a lower temperature.

TABLE III.

SHOWING GERMICIDAL ACTION OF MILK OF LIME UPON FRESH CANE JUICE AT VARIOUS TEMPERATURES

| Strength of Germicide | Temp. | Dilution of Plate | Average No. of Colonies | (+ 000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|---|
| Milk of Lime 5%....... | 25° C | 1:1000 | 48 | 48 | 92.7 | .351 |
| Control................ | 25° C | 1:1 mil. | 33 | 33,000 | .... | .... |
| Milk of Lime 5%....... | 35° C | 1:1000 | 25 | 25 | 96.5 | .497 |
| Control................ | 35° C | 1:1 mil. | 35 | 35,000 | .... | .... |
| Milk of Lime 5%....... | 45° C | 1:1000 | 11 | 11 | 98.25 | .635 |
| Control................ | 45° C | 1:1 mil. | 31 | 31,000 | .... | .... |
| Milk of Lime 5%....... | 55° C | 1:1000 | 8 | 8 | 98.35 | .214 |
| Control................ | 55° C | 1:1 mil. | 24 | 24,000 | .... | .... |

Period of Exposure 15'.
Period of Incubation 72 hrs.

From the above table it will be seen that the accelerative influence of elevated temperatures upon the germicidal action of milk of lime was greater than was true of formaldehyde. A rise of thirty degrees produced an increased efficiency of 5.6%, as compared to 2.1% for formaldehyde. The increase in germicidal efficiency is greatest from 25-35°C. Under the head of formaldehyde coefficient it will be seen that milk of lime more nearly approaches formaldehyde in germicidal value at 45°C., at which point its coefficient is 0.635. It is to be expected that the efficiency of every germicide increases with the temperature at which it acts. The fact that their maximum formaldehyde coefficient and their maximum efficiency are not always reached at the same temperature is due to the different rate at which the germicidal action of formaldehyde is accelerated at various temperatures. Thus, at 45°C., milk of lime has an efficiency of 98.25, as compared to 98.88 for formaldehyde, while at 55 the action of the former is only increased to 98.35, while the latter attains an efficiency of 99.65.

TABLE IV.

SHOWING GERMICIDAL ACTION OF SODIUM BISULPHITE UPON FRESH JUICE AT VARIOUS TEMPERATURES.

| Strength of Germicide | Temp. | Dilution of Plate | Average No. of Colonies | (+ 000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|---|
| Sodium Bisulphite 5%.. | 25° C | 1:1000 | 477 | 477 | .... | .0106 |
| Control.................. | 25° C | 1:1 mil. | 10 | 10,000 | .... | .... |
| Sodium Bisulphite 5%.. | 35° C | 1:1000 | 447 | 447 | .... | .0074 |
| Control.................. | 35° C | 1:1 mil. | 10 | 10,000 | .... | .... |
| Sodium Bisulphite 5%.. | 45° C | 1:1000 | 173 | 173 | 13.50 | .0130 |
| Control.................. | 45° C | 1:1 mil. | 10 | 10,000 | .... | .... |
| Sodium Bisulphite 5%.. | 55° C | 1:1000 | 105 | 105 | 42. | .00615 |
| Control.................. | 55° C | 1:1 mil. | 9 | 9,000 | .... | .... |

Period of Exposure 15′.
Period of Incubation 48 hrs.

From the above table it will be seen that sodium bisulphite has a comparatively weak germicidal action even at elevated temperatures. At temperatures below 45°C. its efficiency, as derived from the formula, is negative. A very striking fact in connection with the effect of elevated temperatures upon the germicidal action is shown in the increase of efficiency produced by a rise in temperature from 25 to 35°C. From a negative efficiency at 25, it reached 13.5 at 45, and is more than tripled by an increase of ten degrees from 45 to 55. We might regard 35°C. as the critical temperature for the germicidal efficiency of sodium bisulphite. No doubt this difference is to be attributed to the greater liberation of $SO_2$ at this temperature.

## TABLE V.

SHOWING GERMICIDAL ACTION OF SODIUM FLUORIDE UPON FRESH JUICE AT VARIOUS TEMPERATURES.

| Strength of Germicide | Temp. | Dilution of Plate | Average No. of Colonies | (+ 000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|---|
| Sodium Fluoride 5%.... | 25° C | 1:1000 | 333 | 333 | .... | .0228 |
| Control................ | 25° C | 1:1 mil. | 15 | 15,000 | .... | .... |
| Control................ | 25° C | 1:40,000 | 370 | 14,800 | .... | .... |
| Sodium Fluoride 5%.... | 35° C | 1:1000 | 393 | 393 | .... | .0126 |
| Control................ | 35° C | 1:1 mil. | 14 | 14,000 | .... | .... |
| Sodium Fluoride 5%.... | 45° C | 1:1000 | 343 | 343 | .... | .00723 |
| Control................ | 45° C | 1:1 mil. | 11 | 11,000 | .... | .... |
| Sodium Fluoride 5%.... | 55° C | 1:1000 | 20 | 20 | 90.95 | .0394 |
| Control................ | 55° C | 1:1 mil. | 10 | 11,000 | .... | .... |

From the above table it will be noted that Sodium Fluoride has a negative germicidal efficiency as measured by the formula $E = 100 - \left(\frac{10}{\frac{1}{S}} \times \frac{L}{1}\right)$ at temperature under 55° C. The acceleration of efficiency between 45° C and 55° C is very striking.

Period of Exposure 15'.
Period of Incubation 72 hrs.

## TABLE VI.

SHOWING GERMICIDAL ACTION OF CHLORIDE OF LIME UPON FRESH JUICE AT VARIOUS TEMPERATURES.

| Strength of Germicide | Temp. | Dilution of Plate | Average No. of Colonies | (+ 000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|---|
| 2% Chloride of Lime.... | 25° C | 1:1000 | 18 | 18 | 91.0 | .283 |
| Control................ | 25° C | 1:1 mil. | 4 | 4,000 | .... | .... |
| 2% Chloride of Lime.... | 35° C | 1:1000 | 9 | 9 | 95.56 | .398 |
| Control................ | 35° C | 1:1 mil. | 4 | 4,000 | .... | .... |
| 2% Chloride of Lime.... | 45° C | 1:1000 | 7 | 7 | 96.5 | .321 |
| Control................ | 45° C | 1:1 mil. | 4 | 4,000 | .... | .... |
| 2% Chloride of Lime.... | 55° C | 1:1000 | 4 | 4 | 98.0 | .178 |
| Control................ | 55° C | 1:1 mil. | 4 | 4,000 | .... | .... |

It will be seen from the above table that Chloride of Lime is highly efficient as a germicide even at low temperatures. Its maximum formaldehyde coefficient is attained at 35° C. Its maximum efficiency was 98.0, which was reached at 55° C.

Period of Exposure 15'.
Period of Incubation 72 hrs.

## Table VII.

SHOWING GERMICIDAL ACTION OF AMMONIUM FLUORIDE AT VARIOUS TEMPERATURES.

| Strength of Germicide | Temp. | Dilution of Plate | Average No. of Colonies | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|---|
| Ammonium Fluoride 5% | 25° C | 1:1000 | 19 | 19 | 84.2 | .161 |
| Control................ | 25° C | 1:1 mil. | 6 | 6,000 | .... | .... |
| Ammonium Fluoride 5% | 35° C | 1:1000 | 13 | 13 | 90.75 | .191 |
| Control................ | 35° C | 1:1 mil. | 7 | 7,000 | .... | .... |
| Ammonium Fluoride 5% | 45° C | 1:1000 | 7 | 7 | 95.0 | .225 |
| Control................ | 45° C | 1:1 mil. | 7 | 7,000 | .... | .... |
| Ammonium Fluoride 5% | 55° C | 1:1000 | 5 | 5 | 95.0 | .071 |
| Control................ | 55° C | 1:1 mil. | 5 | 5,000 | .... | .... |

It will be noticed from the above table that Ammonium Fluoride differs from the other germicides in the fact that its maximum efficiency is attained at 45° C. Its maximum formaldehyde coefficient is attained at this temperature also.

Period of Exposure 15′.
Period of Incubation 72 hrs.

## Table VIII.

SUMMARIZING INFLUENCE OF TEMPERATURE AND LENGTH OF EXPOSURE UPON THE EFFICIENCY AND FORMALDEHYDE COEFFICIENTS OF VARIOUS GERMICIDES.

| Germicide | Influence of Temperature upon Efficiency | | | | Influence of Length of Exposure | |
|---|---|---|---|---|---|---|
| | Max. Eff. | Temp. for Max. Eff. | Max. Form. Coeff. | Temp. for Max. Form. Coeff. | Max. Form. Coeff. | Time for Max. Form. Coeff. |
| Formaldehyde, 0.5%........ | 99.65 | 55 | ... | ... | ... | ... |
| Chloride of Lime, 2%...... | 98.0 | 55 | .398 | 35 | .375 | 20′ |
| Ammonium Fluoride, 5%... | 95.0 | 45 | .225 | 45 | .225 | 30′ |
| Milk of Lime, 5%.......... | 98.35 | 55 | .633 | 45 | .615 | 45′ |
| Sodium Fluoride, 5%....... | 90.95 | 55 | .0394 | 55 | .0287 | 20′ |
| Sodium Bisulphite, 5%...... | 42.0 | 55 | .0130 | 45 | .0198 | 20′ |

Referring to the above table, it will be noted that ammonium fluoride alone of all the germicides tested reaches its maximum efficiency at a temperature lower than the maximum temperatures employed in the experiments. While with this exception the maximum efficiency of all the germicides coincides with the maximum temperature employed, yet their formaldehyde coefficient reaches its maximum in almost every case at the lower temperatures. As has been previously pointed out, this is due to the fact that the rate of acceleration of the germicidal action of formaldehyde by elevations in temperatures does not coincide with that produced on the other germicides. As a result of this different rate of acceleration, we have a maximum efficiency almost invariably at the maximum temperature, but a maximum formaldehyde coefficient at the lower temperature. It will be noted in the above table that the rank taken by the various germicides, as based upon their relative maximum efficiency, is different from that shown in Table I. This is due to the fact that the comparison in that table was made at room temperature, while here their relative efficiency is compared at 45°C. and 55°C.

The data shown in the column headed "Influence of Length of Exposure" was obtained from a series of special experiments. It was thought probable that the comparative efficiency of the weaker germicides and formaldehyde might increase with the length of exposure. In most of the cases, however, the maximum formaldehyde coefficient was attained in the period of shortest exposure. Milk of lime is apparently an exception to this rule, and is more nearly comparable with formaldehyde in its efficiency in a 45′ exposure.

## COMPARISON OF EFFICIENCY OF GERMICIDES UPON FERMENTED JUICES.

We have previously referred to the fact that the efficiency of a germicide varies not only with the various species of micro-organisms upon which it may be tested, but also with the composition of the substance with which it must be brought in contact to perform its work. Our previous experiments were conducted upon fresh juices only. That the efficiency of the germicides might be quite different when tested upon fermented juices might easily be assumed upon the grounds stated above. Not only is the composition of the substance different in the two cases, but the species of microorganisms predominating may also differ, which would further influence the efficiency of the germicides. It was considered necessary, therefore, to test the germicides upon fermented juices, in order to ascertain whether their efficiency was the same under these conditions as when used on fresh juices. For these experiments two types of fermented juices were selected, which represented two stages of fermentation. The partly fermented juice was one which had been allowed to ferment for twelve hours in the incubator, while the badly fermented juice was one which had been kept on hand for some time in the laboratory and was completely fermented. The method of treating them with the germicidal agents was the same as that previously described.

TABLE IX.

SHOWING COMPARATIVE EFFICIENCY OF FORMALDEHYDE UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Formaldehyde | Control | Formaldehyde | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 120 | 7,000 | 1,220 | 140,000 |
| 2 | 80 | 8,000 | 1,200 | 140,000 |
| 3 | 80 | 12,000 | 1,040 | 120,000 |
| 4 | 80 | 9,000 | 1,220 | 112,000 |
| 5 | 100 | 7,000 | 1,100 | 140,000 |
| 6 | 80 | 7,000 | 1,160 | 160,000 |
| Ave. | 90,000 | 8,000 | 1,157,000 | 135,000,000 |

Efficiency of Slightly Fermented Juice............. 94.40
Efficiency on Badly Fermented Juice............... 95.75
Dilution of Control.............................. 1:1 million
Dilution of Formaldehyde Treated Samples........ 1:1000

It will be noted from the above table that the germicidal efficiency of formaldehyde is greater on badly fermented juices than upon juice that is only partly fermented. This is perhaps due to the fact that in the former there is a greater preponderance of less resistant species of microorganisms, such as yeast and non-sporulating bacteria. If we compare the germicidal efficiency of formaldehyde on fermented juice with that derived in our experiments on fresh juice, we find, however, that its germicidal action does seem to be impaired by the products formed during fermentation. Referring to Table I, we find that formaldehyde has a germicidal efficiency on fresh juice of 98.85, while on badly fermented juice its efficiency is only 95.71. This decrease in germicidal efficiency may be due to one or to a combination of several causes. It may be due to certain combinations that the formaldehyde forms with the products of fermentation. The mere excessive numbers of microorganisms in the badly fermented juice might of itself tend to lower the germicidal efficiency of a substance. Since the action of most germicides depends upon a definite reaction taking place between them and the microorganisms, which they kill, it is evident that the greater the number of microorganisms the greater will be the quantity of germicide required. There are, of course, certain exceptions to this rule, for some germicides act as catalytic poison, which kill by mere contact and do not depend upon forming chemical combinations for their germicidal action. Prominent among this class of germicides are chloroform, ether, carbon bisulphide, chloral, etc. Formaldehyde, however, like all aldehydes, owes its germicidal action to the ease with which it reacts with the cell substance of microorganisms. It acts readily upon albumen, forming insoluble compounds. According to Loew (1), the toxicity of all aldehydes is directly proportionate to their instability, the most unstable ones having the greatest toxicity.

(1) Ein Naturliches System der Giftwirkungen.

TABLE X.

SHOWING COMPARATIVE EFFICIENCY OF CHLORIDES OF LIME UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Chloride of Lime 2% | Control | Chloride of Lime 2% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 20 | 7,000 | 27 | 140,000 |
| 2 | 18 | 8,000 | 20 | 140,000 |
| 3 | 18 | 12,000 | 20 | 120,000 |
| 4 | 17 | 9,000 | 30 | 112,000 |
| 5 | 17 | 7,000 | 23 | 140,000 |
| 6 | 18 | 7,000 | 29 | 160,000 |
| Ave. | 18,000 | 8,000,000 | 25,000 | 135,000,000 |

| | |
|---|---|
| Efficiency Slightly Fermented Juice | 95.5 |
| Formaldehyde Coefficient | 1.24 |
| Efficiency Badly Fermented Juice | 99.63 |
| Formaldehyde Coefficient | 11.5 |
| Dilution of Control | 1 :1 million |
| Dilution of Treated Sample | 1 :1000 |

The increased germicidal efficiency of chloride of lime when acting upon sour juices is very striking. This significant increase in germicidal efficiency under these conditions is most probably due to the greater liberation of chlorine from the chloride of lime by the action of the acids developed in the course of the fermentation of the juice. In this case, however, just as in the case of formaldehyde, the greater efficiency upon fermented juice is, no doubt, in part due to the preponderance of species of low resistance in the badly fermented juice. In both of the fermented juices chloride of lime is superior to formaldehyde. Its formaldehyde coefficient on the badly fermented juice is 11.5, which means that under these conditions chloride of lime is much superior to formaldehyde as a germicide.

## Table XI.

SHOWING COMPARATIVE EFFICIENCY OF SODIUM BISULPHITE UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Sodium Bisulphite 5% | Control | Sodium Bisulphite 5% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 960 | 7,000 | 760 | 140,000 |
| 2 | 800 | 8,000 | 740 | 140,000 |
| 3 | 620 | 12,000 | 660 | 120,000 |
| 4 | 760 | 9,000 | 600 | 112,000 |
| 5 | 720 | 7,000 | 600 | 140,000 |
| 6 | 900 | 7,000 | 800 | 160,000 |
| Ave. | 793,000 | 8,000,000 | 694,000 | 135,000,000 |

| | |
|---|---|
| Efficiency Slightly Fermented Juice................ | .... |
| Formaldehyde Coefficient........................ | 0.0113 |
| Efficiency Badly Fermented Juice.................. | 74.3 |
| Formaldehyde Coefficient.......................... | 0.166 |
| Dilution of Control................................ | 1:1 million |
| Dilution of Treated Sample........................ | 1:1000 |

It will be noted from the above table that the germicidal efficiency of sodium bisulphite is greater with badly fermented juices than with the slightly fermented. Compared with its germicidal efficiency on fresh juice, as shown in Table I, it is almost twice as efficient. The greater efficiency of sodium bisulphite on fermented juices is to be attributed to a greater liberation of sulphur dioxide in an acid solution. The same explanation doubtless applies in this case as in the case of chloride of lime.

## Table XII.

SHOWING COMPARATIVE EFFICIENCY OF AMMONIUM PLUORIDE UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Ammonium Fluoride 5% | Control | Ammonium Fluoride 5% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 10 | 14,000 | 220 | 60,000 |
| 2 | 10 | 13,000 | 200 | 77,000 |
| 3 | 10 | 10,000 | 140 | 78,000 |
| 4 | 7 | 12,000 | 180 | 71,000 |
| 5 | 7 | 15,000 | 160 | 66,000 |
| 6 | 10 | 12,000 | 140 | 79,000 |
| Ave. | 9,000 | 13,000,000 | 173,000 | 71,000,000 |

Efficiency Slightly Fermented Juice.............. 96.54
Formaldehyde Coefficient........................ 1.61
Efficiency Badly Fermented Juice................. 87.85
Formaldehyde Coefficient........................ 0.353
Dilution of Control............................. 1:1 million
Dilution of Treated Sample...................... 1:1000

It is apparent from the above table that the germicidal efficiency of ammonium fluoride is materially impaired by contact with badly fermented juice. Its germicidal efficiency on slightly fermented juice, however, is greater than on fresh juice, as shown in Table I.

### Table XIII.

SHOWING COMPARATIVE EFFICIENCY OF SODIUM FLUORIDE UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Sodium Fluoride 5% | Control | Sodium Fluoride 5% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 620 | 14,000 | 3,300 | 60,000 |
| 2 | 700 | 13,000 | 2,000 | 77,000 |
| 3 | 700 | 10,000 | 5,000 | 78,000 |
| 4 | 620 | 12,000 | 3,500 | 71,000 |
| 5 | 800 | 15,000 | 3,500 | 66,000 |
| 6 | 600 | 12,000 | 2,500 | 79,000 |
| Ave. | 673,000 | 13,000,000 | 3,300,000 | 71,000,000 |

Efficiency Slightly Fermented Juice................ ....
Formaldehyde Coefficient.......................... .0214
Efficiency Badly Fermented Juice.................. ....
Formaldehyde Coefficient.......................... .0184
Dilution of Control............................... 1:1 million
Dilution of Treated Sample........................ 1:1000

From the above table it will be noted that sodium fluoride, like ammonium fluoride, has a lower germicidal efficiency on fermented juice than on fresh juice.

TABLE XIV.

SHOWING COMPARATIVE EFFICIENCY OF BISULPHIDE OF LIME UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Bisulphite of Lime 5% | Control | Bisulphite of Lime 5% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 660 | 14,000 | 780 | 60,000 |
| 2 | 680 | 13,000 | 480 | 77,000 |
| 3 | 680 | 10,000 | 660 | 78,000 |
| 4 | 520 | 12,000 | 640 | 71,000 |
| 5 | 640 | 15,000 | 620 | 66,000 |
| 6 | ... | 12,000 | ... | 79,000 |
| Ave. | 636,000 | 13,000,000 | 636,000 | 71,000,000 |

| | |
|---|---|
| Efficiency Slightly Fermented Juice................ | .... |
| Formaldehyde Coefficient......................... | .0224 |
| Efficiency Badly Fermented Juice.................. | 55.25 |
| Formaldehyde Coefficient......................... | .0956 |
| Dilution of Control.............................. | 1 :1 million |
| Dilution of Treated Sample....................... | 1 :1000 |

From the above table it will be seen that calcium bisulphite, like sodium bisulphite, has a higher germicidal efficiency on badly fermented juice than on slightly fermented juice. The explanation of this is the same as was stated in the former case, viz., that there is a greater liberation of sulphur dioxide in the more acid solution. Comparing the germicidal efficiency of the two bisulphites, we find their relative efficiency to be almost in direct proportion to their $SO_2$ content.

## Table XV.

SHOWING COMPARATIVE EFFICIENCY OF MILK OF LIME UPON FERMENTED JUICES.

| Plate | Slightly Fermented Juice | | Badly Fermented Juice | |
|---|---|---|---|---|
| | Milk of Lime 5% | Control | Milk of Lime 5% | Control |
| | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. | (+ 000) No. of Micro. per c. c. |
| 1 | 58 | 14,000 | 66 | 60,000 |
| 2 | ... | 13,000 | 75 | 77,000 |
| 3 | 50 | 10,000 | 74 | 78,000 |
| 4 | 50 | 12,000 | 60 | 71,000 |
| 5 | 60 | 15,000 | 54 | 66,000 |
| 6 | 50 | 12,000 | 81 | 79,000 |
| Ave. | 54,000 | 13,000,000 | 68,000 | 71,000,000 |

| | |
|---|---|
| Efficiency Slightly Fermented Juice | 79.25 |
| Formaldehyde Coefficient | 0.270 |
| Efficiency Badly Fermented Juice | 95.25 |
| Formaldehyde Coefficient | 0.896 |
| Dilution of Control | 1:1 million |
| Dilution of Treated Sample | 1:1000 |

Milk of lime also seems to have a higher germicidal efficiency on fermented juice than on fresh juice. Comparing its efficiency on badly fermented juice, as shown in the above table, with that given in Table I, we have an efficiency of 95.21 in the former, as against 86.2 in the latter.

### THE COMPARATIVE EFFICIENCY OF GERMICIDES UNDER CONDITIONS WHICH INVOLVE THEIR PENETRATIVE POWERS.

Some germicides which are highly efficient under conditions which do not require any power of penetration on their part in order for them to come in immediate contact with the micro-organisms to be destroyed are relatively impotent under conditions which involve such properties. On account of this fact, we often find that the most powerful germicides can sometimes be substituted to advantage in such cases by ones that are weaker under ordinary conditions. As a result of this variation in penetrative power on the part of germicides, we find that some of the less powerful ones are strongly recommended for cleaning vats and tanks in distilleries. In such cases it is highly important for the germicide to have the power of penetrating the minute crevices in the surfaces of the vats, in order for their germicidal powers to reach the microorganisms contained therein. It is obvious that the conditions under which our previous experiments upon the efficiency of germicides on juices were conducted are not strictly comparable to sugar house conditions, if we consider the importance of the penetrating power of these substances. In the previous experiments the juice and germicides were mixed in volumetric flasks. Under these conditions the inner surfaces of the container were so smooth as to offer no difficulties for a thorough contact to be established between germicide and juice at the sides of the container. Such ideal conditions do not obtain in the apparatus of sugar factories, where the unevenness of the inner surfaces furnishes favorable points for the development of microorganisms where they are not easily reached by germicides. In order to test the efficiency of the germicides under conditions that more closely approximate those in the sugar factory, the following experiments were conducted. Instead of treating the juice in flasks, two-gallon wooden buckets were used for the purpose. One liter of fermented juice was poured into each bucket and allowed to remain for thirty minutes before applying the germicide. Two hundred ccs of the germicide solution was then poured into the bucket, and the contents then thoroughly mixed by stirring. To the bucket containing the control juice two hundred ccs of sterile water was added and mixed, as in the other cases. After the germicides had remained in contact with the juice for fifteen minutes, the contents of the buckets were poured off and the buckets were inverted and allowed to drain for two minutes. They were then turned up in their normal position and 500 ccs of sterile water added to each bucket and thoroughly mixed with the contents that had adhered to the sides. After the insides of each bucket had been thoroughly washed down, transfers were made to flasks of sterile water and plates made from them in the usual way. Although there are relatively large factors of error in an experiment of this kind, it was thought that the results would show, in a general way, the facts that were intended to be brought out. One of the largest factors of error would naturally be the variations in the actual amount of juice left in each bucket and the different degrees of dilution that the addition of the 500 ccs of water would represent in each case. The opportunity for the buckets to drain were identical in each case, so far as the time allowed for this purpose and the position of the buckets were concerned. The same juice was used for each bucket, so the rate at which it would drain from the sides should have been apparently the same in each case, except that the viscosity of the juice might have varied with the addition of the various germicides.

TABLE XVI.

SHOWING COMPARATIVE EFFICIENCY OF GERMICIDES UPON JUICES IN WOODEN PAILS.

| Series Disinfectant | % Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|
| Control A | ... | 18 | 3,600+ | .... | .... |
| Control B | ... | 14 | 2,800+ | .... | .... |
| Formaldehyde | 0.5% | 73 | 73+ | 88.60 | 1. |
| Sodium Bisulphite | 5.0% | 39 | 39+ | 39.50 | 0.188 |
| Milk of Lime | 5.0% | 13 | 13+ | 79.70 | 0.561 |
| Chloride of Lime | 2.0% | 8 | 8+ | 95.00 | 2.08 |
| Ammonium Fluoride | 5.0% | 4 | 4+ | 93.75 | 1.82 |
| Sodium Fluoride | 5.0% | 198 | 198+ | .... | 0.362 |

Time allowed to act .......... 15′
Incubation Period .......... 72 hrs.
Dilution of Control .......... 1:200,000
Dilution of Treated Sample .......... 1:100,000

The above table shows that the germicidal efficiency of the various substances is quite different under the conditions of this experiment. Under these conditions it appears that both ammonium fluoride and chloride of lime have a higher germicidal efficiency than formaldehyde. Formaldehyde shows a comparatively low germicidal efficiency in this experiment, which is no doubt to be attributed to its lack of penetrative power, and also, but to a lesser extent, to its lower efficiency on fermented juice. Chloride of lime has a formaldehyde efficiency of 2 in this experiment, which indicates that where the penetrative power of the germicide is involved this substance is superior to formaldehyde. It is perhaps on account of the penetrative power of chloride of lime that it is so strongly recommended for cleaning filter bags in sugar factories.

TABLE XVII.

SHOWING COMPARATIVE EFFICIENCY OF GERMICIDES UPON JUICES IN WOODEN PAILS.

| Disinfectant Used | % Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|
| Formaldehyde .............. | 0.5% | 196 | 196+ | 95.5 | 1. |
| Sodium Bisulphite........... | 5.0% | 212 | 212+ | 51.4 | 0.0925 |
| Chloride of Lime............ | 2.0% | 19 | 19+ | 98.26 | 2.58 |
| Milk of Lime............... | 5.0% | 17 | 17+ | 96.10 | 1.15 |
| Sodium Fluoride............ | 5.0% | 110 | 110+ | 79.4 | 0.218 |
| Ammonium Fluoride........ | 5.0% | 180 | 180+ | 58.75 | 0.109 |
| Control ................... | ... | 212 | 21,200+ | .... | .... |
| Control.................... | ... | 112 | 22,400+ | .... | .... |

Time allowed to act............. 15′
Incubation Period................ 72 hrs.
Dilution of Control.............. 1:1,000,000 and 1:200,000
Dilution of Treated Sample....... 1:1000

The juice used in the above experiment was quite different from that employed in the previous case. This juice was not so completely fermented, but was very viscous as a result of a vigorous dextran fermentation that had taken place in it. As a result of this greater viscosity of the juice it would seem that the efficiency of the germicides in this case would be even more dependent upon their penetrative power than in the previous experiment. In this case the juice would adhere to the sides more tenaciously, and it would be more difficult for the germicides to come in through contact with it.

The results of this experiment are somewhat different from those obtained in the preceding one. In this case formaldehyde is surpassed in germicidal efficiency by chloride of lime and milk of lime. The formaldehyde coefficient of the former is very nearly the same in both experiments. The germicidal efficiency of formaldehyde seems to be higher in this experiment than in the preceding one. From these experiments it seems probable that formaldehyde can well be dispensed with in favor of chloride of lime as a germicide for use in certain special cases in the cleaning of sugar factories. In order, however, to ascertain the relative expense connected with the use of the two substances, their relative germicidal efficiency must be taken in connection with the prices at which they can be procured. This comparison will be taken up in a subsequent chapter.

THE STIMULATIVE ACTION OF SUBLETHAL APPLICATIONS OF VARIOUS GERMICIDES UPON THE MICROORGANISMS OF CANE JUICE.

The theory that cell development may be stimulated by the application of toxic substances in quantities insufficient to produce death was first proposed as a biological law by Arndt ( [1] ) in his work, "Biologische Studien." The application of this law to microorganisms has been conclusively established by the investigations of Hüne ( [2] ), Koch ( [3] ), Fred ( [4] ), Schulze ( [5] ), Raulin ( [6] ), Nageli ( [7] ), Kruger ( [8] ) and others. In a recent investigation by Meade ( [9] ) the application of this law to the microorganisms in cane sugar products has been very clearly demonstrated. As Meade relied solely upon chemical analyses to determine the relative stimulative power of various germicides, it seemed that the duplication of a part of his work by bacteriological instead of chemical methods might be of some scientific value. Since only a few of the germicides used in this investigation were tested by the author above referred to, the following experiments are not an entire duplication of the previous work.

The investigation of the stimulative action of the various germicides upon the microorganisms in cane juice is warranted in the scope of the present work solely upon the grounds that it possesses scientific interest. It is not calculated to yield results that are susceptible of any practical application in connection

(1) Biologische Studien I.
(2) Cent. fur Bakt. Abt. I. Bd. 48, 1909.
(3) Cent. fur Bakt. Abt. II. Bd. 31.
(4) Cent. fur Bkt. Abt. II. Bd. 31.
(5) Pflugers Archives. Bd. 42, 1888.
(6) These, Paris, 1810.
(7) Schweizer Gesellsch, f. d. gesamt. Naturwissenschaft. Bd. 33, 1893.
(8) Cent. fur Bakt. Abt. II. Bd. I, p. 10.
(9) Action of Disinfectants on Sugar Solutions. La. Planter, 1912, p 7349.

with the use of germicides in sugar factories. The demonstration by Meade of the stimulative action of sugar factory germicides was quite accidental, since he was primarily investigating the relative value of different substances as preservatives for laboratory samples when he observed that his treated samples deteriorated more rapidly than the controls.

The method of conducting the experiments was as follows: The juice and germicides were mixed, as in the previous experiments. After a period of exposure of fifteen minutes, the juice was transferred to dilution flasks, plated out and incubated for one week. A comparison of the untreated juice and those portions of it which had been treated with the germicides showed the initial depressing action of the treatment. The flasks containing the treated juices were then incubated for twenty-four hours at a temperature of 35°C., at the expiration of which time they were again plated out and compared with an untreated control.

TABLE XVIII.

PRELIMINARY EXPERIMENT UPON THE STIMULATIVE ACTION OF SMALL QUANTITIES OF GERMICIDE UPON THE MICROORGANISMS OF CANE JUICE.

| Germicide | 15 Minutes After Addition of Germicide | | | After 24 Hours | |
|---|---|---|---|---|---|
| | Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Average No. of Colonies | (+000) No. of Micro. per c. c. |
| Control .................. | ........ | 41 | 8,200 | 167 | 167,000 |
| Formaldehyde.......... ...... | 1:50,000 | 24 | 4,800 | 60 | 60,000 |
| Milk of Lime.............. | 1:200 | 11 | 2,200 | 5 | 1,000 |
| Chloride of Lime........... | 1:5,000 | 13 | 2,600 | 84 | 16,800 |
| Ammonium Floride......... | 1:5,000 | 25 | 5,000 | 97 | 19,400 |
| Sodium Bisulphite.......... | 1:200 | 45 | 9,000 | 10 | 2,000 |
| Calcium Bisulphite......... | 1:200 | 29 | 5,800 | 8 | 1,600 |
| Sodium Fluoride........... | 1:200 | 25 | 5,000 | 20 | 4,000 |

Dilution of Control............. 1:200,000 and 1:1 million
Dilution of Treated Sample..... 1:200,000 and 1:200,000

The above table shows the results of a preliminary experiment, the purpose of which was to ascertain the strengths in which the various germicides produce a stimulative action. It will be observed from this table that the strengths of the germicides were too great, and that the number of microorganisms in the control flask exceeded those in the treated samples.

Meade ( [1] ) found formaldehyde to be stimulative upon the microorganisms in raw sugar washings when used in the strength of 1:50,000. However, this dilution was made on the basis of formalin, which contains 40% formaldehyde, thus representing a formaldehyde dilution of 1:125,000.

TABLE XIX.

SHOWING THE STIMULATIVE ACTION OF SMALL QUANTITIES OF GERMICIDES UPON THE MICROORGANISMS OF CANE JUICE.

| Germicide | 15 Minutes After Addition of Germicide | | | After 24 Hours | |
|---|---|---|---|---|---|
| | Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Average No. of Colonies | (+000) No. of Micro. per c. c. |
| Control ................ | ........ | 21 | 4,200 | 47 | 47,000 |
| Formaldehyde............ | 1:100,000 | 22 | 4,400 | 81 | 81,000 |
| Milk of Lime............ | 1:1,000 | 24 | 4,800 | 27 | 27,000 |
| Chloride of Lime.......... | 1:20,000 | 18 | 3,600 | 135 | 135,000 |
| Ammonium Fluoride....... | 1:20,000 | 19 | 3,800 | 85 | 85,000 |
| Sodium Bisulphide......... | 1:1,000 | 23 | 4,600 | 46 | 46,000 |
| Calcium Bisulphite......... | 1:1,000 | 21 | 4,200 | 73 | 73,000 |
| Sodium Fluoride........... | 1:1,000 | 30 | 6,000 | 14 | 14,000 |

Dilution of Control............ 1:200,000 and 1:1 million
Dilution of Treated Sample..... 1:200,000 and 1:1 million

In the above table it will be noted that formaldehyde 1:100,000, chloride of lime 1:20,000 and ammonium fluoride 1:20,000 exerted a marked stimulative action upon the microorganisms of cane juice. The degree of stimulation by chloride of lime is especially marked. The results in this experiment approximately agree with those of Meade, which showed the maximum stimulative strength of formaldehyde to be 1:125,000, chloride of lime from 1:10,000 to 1:20,000, and the same for ammonium fluoride. Of the remaining germicides only bisulphite of lime showed any stimulative action. Sodium bisulphite did not seem to decrease the number of organisms in the juice to any appreciable extent. It is interesting to note that only the stronger germicides seemed capable of exercising a stimulative action upon the microorganisms in juices.

## Chapter III.

### EXPERIMENTS UPON SYRUPS AND SUGARS.

We have already observed that the efficiency of germicides is influenced by the composition of the substance upon which they are used. Since the composition of syrups and sugars present very different conditions for the action of germicides from those obtained in our previous experiments, it is necessary to compare the efficiency of the germicides under these conditions. These substances differ from juices not only in their composition but also in the microorganisms that they contain. In cane juices the predominant species are usually of low resistance to heat and to germicidal agents. The predominant species in sugars and syrups are, on the contrary, spore forming bacteria that are highly resistant to heat and to germicides. In order to test the efficiency of the various germicides upon these products the following experiments were conducted: A syrup which was badly fermented was selected for the experiments. It had an original density of 74 Brix, which had decreased to 65 at the time of the experiment. The method of treating it was the same as that described for the treatment of juices.

TABLE XX.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON CANE SYRUP (FERMENTING SYRUP AT 15° C.).

| Substance Employed | % Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|
| Control | ... | 702 | 702,000 | .... | .... |
| Formaldehyde | 0.5% | 13 | 13,000 | 90.75 | 1. |
| Sodium Bisulphide | 10.0% | 72 | 72,000 | .... | 0.009 |
| Chloride of Lime | 2.0% | 11 | 11,000 | 68.8 | 0.296 |
| Milk of Lime | 5.0% | 17 | 17,000 | .... | 0.0764 |
| Sodium Fluoride | 5.0% | 131 | 131,000 | .... | 0.0099 |
| Ammonium Fluoride | 5.0% | 12 | 12,000 | 14.5 | 0.108 |
| Sulphite of Lime | 5.0% | 15 | 15,000 | .... | 0.086 |

Period of Exposure ............ 15′
Period of Incubation ............ 72 hrs.
Dilution of Plates ............ 1:1000

From the above table it will be seen that all of the germicides have a comparatively low efficiency on syrups. Only three of the germicides showed a positive efficiency, and the formaldehyde coefficients are low in every case. The efficiency of formaldehyde, although far greater than any of the others, is very low in comparison with the results obtained on juice.

For the experiments on sugar a badly deteriorated sugar was selected and a fifty per cent solution made with water. The method of treating with the various germicides was the same as in the previous experiments.

Table XXI.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON DETERIORATED SUGAR.

| Germicides | % Strength | Average No. of Colonies | (+000) No. of Micro. per c. c. | Efficiency | Formaldehyde Coefficient |
|---|---|---|---|---|---|
| Control | ... | 48 | 48,000 | .... | .... |
| Formaldehyde | 0.5% | 8 | 8,000 | .... | 1. |
| Chloride of Lime | 2% | 9 | 9,000 | .... | 0.221 |
| Ammonium Fluoride | 5% | 5 | 5,000 | .... | 0.159 |
| Calcium Bisulphite | 5% | 15 | 15,000 | .... | 0.532 |
| Sodium Bisulphite | 10% | 25 | 25,000 | .... | 0.0159 |
| Sodium Fluoride | 5% | 14 | 14,000 | .... | 0.0570 |
| Milk of Lime | 5% | 10 | 10,000 | .... | 0.0798 |

Period of Exposure .................. 15'
Period of Incubation .................. 72 hrs.
Dilution of Plates .................. 1:1000

In this experiment only formaldehyde showed a positive efficiency, and it was exceedingly low. The formaldehyde coefficients were without exception very low. An interesting point that developed during the course of these investigations was the variation in the germicide efficiency of formaldehyde on different solutions. In the two tables given above, the germicidal efficiency of formaldehyde, although comparatively low, is much higher than the results obtained from experiments on other samples of syrups and sugars. It appears probable that the low efficiency in the other cases was due to the fact that certain combinations were formed between the formaldehyde and certain bodies contained in the solution. That such combination may take place is suggested by Norris ([1]), who attributes the influence of formaldehyde upon the polarization of sugar solutions to a reaction taking place between it and the sugar. To quote the author on the subject, we have the following: "The most probable explanation of the reaction between the formaldehyde and the sugar seems to the writer to be the formation of an unstable compound of the two, which has a higher polarization than the sugar itself. The formation of such combinations is also suggested in the work of Yoder and Taggart." ([2])

(1) Loc. cit.

(2) Occurrence of Formaldehyde in Sugar House Products. Int. Sugar Jour., 1910.

## Chapter IV.

### THE DETERMINATION OF THE PHENOL CO-EFFICIENT OF THE VARIOUS GERMICIDES.

The usual method for the standardization of disinfectants in public health work is by determining their phenol coefficient. Phenol is selected as a standard for these comparisons owing to the fact that its action is less affected by the presence of organic matter. In order to compare the efficiency of the germicides employed in the preceding experiments, on the same basis as that used in medical investigations, an attempt was made to establish the phenol coefficient for each of the germicides.

There are several methods now in use for the determination of the phenol coefficient of germicides. The most important of these may be briefly described as follows: The Rideal Walker(1) method, which was one of the first employed for this purpose, consists in exposing a 24-hour culture of the test organism to the action of various dilutions of phenol and the germicide to be tested. The phenol coefficient is then derived by dividing the figure indicating the degree of dilution of the disinfectant that kills the organism in a given time by that expressing the degree of dilution of the phenol that kills the same organism in the same time under exactly the same conditions. The method is carried out as follows: Phenol solutions of known strength are used. The cultures are grown in a standard medium, from which transplants are made every twenty-four hours. The inoculations are made with platinum loops of standard size. Usually about four different dilutions of the phenol and the disinfectant to be tested are employed. Five ccs of these dilutions are placed in sterile tubes, to which are added at one-half minute intervals one drop per cc of solution of the culture of the test organism. At the end of two and one-half minutes a loopful of each of the mixtures is transferred to a test tube containing 5 ccs of a standard culture medium, an interval of half a minute being thus allowed for taking the samples from the different solutions. These transfers are repeated at the end of 5, 7½, 10, 12½ and 15 minutes. The inoculated tubes are then incubated for 48 hours, after which the comparisons are made be-

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(1) Journal of the Royal Sanitary Institute, Vol. XXIV, 1903 and 1904, pp. 424-435.

tween the relative strengths of phenol and of the disinfectant, at which growth is prevented. The modification of this method by Anderson and McClintic ( [1] ), which is known as the "Public Health Laboratory Method," consists in comparing the effect of the phenol and disinfectant at 2½ and 15 minutes' exposure, and in extending the period of exposure to thirty minutes instead of fifteen. The number of dilutions employed are also much greater. The Lancet ( [2] ) method differs from the "Public Health Laboratory Method," which is a modification of it, chiefly in the method of making the transfers. In the Lancet method spoons are used instead of platinum loops for transferring the treated culture to the medium.

## METHOD FOR PHENOL COEFFICIENT DETERMINATION:

The accuracy of these determinations depends largely upon the following factors: (1) Temperature of medication; (2) uniformity of age and general condition of the test organisms; (3) uniformity in the proportion of inoculating culture to disinfectant. The temperature at which the test organisms was exposed to the germicide was controlled in our experiments by means of a large bath, which was placed in a wooden box and packed in sawdust. The seeding tubes were submerged beneath the surface of the bath to a depth at which their contents would be entirely below the surface of the surrounding water. Through the top of the box, which was securely fastened, were holes for twenty-one tubes, a thermometer, and one for filling and siphoning off the water. The bath was made according to the description given in Bulletin 82 of the Hygienic Laboratory. It proved very satisfactory, and there was but little difficulty in maintaining the standard temperature at 20°C. throughout the experiments. For sterilizing the platinum loops a fan-tail gas burner was used and a wire rack to hold six of these loops, so that they could be flamed immediately after each transfer. A test tube rack of the required capacity was made from heavy timber by drilling holes of the proper diameter into it to the required depth. The seeding tubes were ¾″ in diameter and

(1) Hygienic Laboratory Bulletin No. 82.
(2) Lancet, London, Vol. 177, Nos. 4498, 4499, 4500.

three inches long. The ones used in the "Public Health Laboratory" were one inch in diameter. Five ccs of the dilutions of the various germicides were placed in these tubes, after they had been sterilized in the autoclave. One-tenth cc of the inoculating culture was introduced into them and the mixture thoroughly shaken. The platinum loops for transferring the culture from the seeding tubes were 4 mm in diameter.

The organism first selected for these experiments was *Bacillus vulgatus*. It was grown in a standard sucrose solution of the same formula as the one used by the author in the investigation of the species occurring in sugar ([1]). Only twenty-four hour cultures were used in the experiments and the incubation period of the tubes, to which the test culture was added, was forty-eight hours. Although it was especially desirable that the tests should be made on this species, there were several factors that militated against successful results. The species forms spores, which are intensely resistant to heat, and is one of the group causing deterioration of sugars. It was thought, therefore, that the results obtained upon its resistance to the different germicides would be especially interesting. It was found, however, to be practically impossible to secure concordant results from the use of this test organism in the experiments. At first the variations in the phenol coefficients in the different experiments were attributed to the influence of gum, which might occur in varying quantities in the different twenty-four hour cultures. The presence of this gum might tend to prevent the germicides from reaching the organism, and in this way the efficiency of the germicide might vary with the amount of gum in the solutions. Filtering off this gum by passing it through a sterile cotton filter, however, gave no better results. It was then discovered that the variations in the results were due to the fact that in some cases spores were present even in the twenty-four hour cultures, and as the strengths of the germicides were entirely insufficient for spore destruction, the germination of these spores and the subsequent growth of their vegetative forms would not be prevented by the highest concentrations of the germicides. The use of this species as a test organism was abandoned and a non-sporulating species substituted for it. The species then

(1) Bulletin 146, La. Experiment Station.

selected was *Bacterium xylinum.* This species is almost invariably found in sugar cane juice, molasses and syrups, where it forms a thick gelatinous membrane known as chitine (1), which has been thoroughly studied by C. A. Browne. It belongs to the acetic ferment group of bacteria. The stock solutions of formaldehyde and phenol used in the experiments were accurately standardized at frequent intervals to determine their strength. The method used for the determination of formaldehyde was the modified hydrogen peroxid method, as given in the official methods (2) of the Bureau of Chemistry. For the standardization of the stock phenol solution the Meissenger and Vortmann (3) iodine method was employed.

TABLE XXII.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.
Proportion of Culture and Germicide, 0.1 c. c. to 5 c. c.
Temperature of Treatment, 20° C.
Culture used, *Bact. xylinum.*

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:150 | — | — | — | | | |
| | 1:175 | × | — | — | | | |
| | 1:200 | × | — | — | — | — | — |
| | 1:250 | × | × | — | — | — | — |
| | 1:300 | × | × | × | × | × | — |
| | 1:400 | × | × | × | × | × | — |
| | 1:500 | × | × | × | × | × | × |
| Phenol | 1:125 | — | — | — | | | |
| | 1:150 | — | — | — | — | | |
| | 1:175 | × | — | — | — | — | — |
| | 1:200 | × | × | — | — | — | — |
| | 1:250 | × | × | × | × | — | — |

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient } \frac{\frac{150}{150} + \frac{250}{250}}{2} = 1$$

(1) The Chemistry of the Sugar Cane and Its Products in Louisiana. Bulletin 91, La. Expt. Station.
(2) Bulletin 107 (revised), p. 33, Bureau of Chemistry, U. S. Dept. Agriculture.
(3) Sutton's Volumetric Analysis, p. 395.

In the above experiment formaldehyde has a phenol coefficient of 1, which is much lower than was anticipated. We should have expected a coefficient of 3 to 3½, judging from the reputed antiseptic values of the two substances.

TABLE XXIII.

SHOWING PHENOL COEFFIENT OF FORMALDEHYDE.
Culture used, *Bact. xylinum.*
Temperature of Treatment, 20° C.
Proportion of Culture and Germicide, 0.1 c. c. to 5 c. c.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:150 | — | — | — | | | |
| | 1:175 | × | — | — | | | |
| | 1:200 | × | × | — | — | — | — |
| | 1:250 | × | × | × | — | — | — |
| | 1:300 | × | × | × | × | × | — |
| | 1:400 | × | × | × | × | × | — |
| | 1:500 | × | × | × | × | × | — |
| Phenol | 1:125 | — | — | — | | | |
| | 1:150 | — | — | — | — | | |
| | 1:175 | × | — | — | — | — | — |
| | 1:200 | × | — | — | — | — | — |
| | 1:250 | × | × | × | × | — | — |
| | 1:300 | × | × | × | × | × | — |

× Indicates growth.
— Indicates no growth.

Phenol Coefficient
$$\frac{\frac{150}{150} + \frac{250}{250}}{2} = 1.$$

The results shown in the above table are the same as obtained in the previous experiment.

## Table XXIV.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.

Culture used, *Bact. xylinum.*
Temperature of Exposure, 20° C.
Proportion of Culture to Germicide, 1 c. c to 5 c. c.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:150 | — | — | — | | | |
| | 1:175 | × | — | — | — | | |
| | 1:200 | × | — | — | — | — | — |
| | 1:250 | × | × | × | × | — | — |
| | 1:300 | × | × | × | × | × | — |
| | 1:400 | × | × | × | × | × | × |
| | 1:500 | × | × | × | × | × | × |
| Phenol | 1:125 | — | — | — | | | |
| | 1:150 | — | — | — | — | | |
| | 1:175 | — | — | — | — | — | — |
| | 1:200 | × | × | — | — | — | — |
| | 1:250 | × | × | × | × | × | × |

× Indicates growth.
— Indicates no growth.

Phenol Coefficient — $\dfrac{\dfrac{150}{175} + \dfrac{250}{200}}{2} = 1.053$

In this experiment formaldehyde has a phenol coefficient of slightly over 1.

## Table XXV.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:150 | — | — | — | | | |
| | 1:175 | — | — | — | — | | |
| | 1:200 | — | — | — | — | — | — |
| | 1:250 | × | — | — | — | — | — |
| | 1:300 | × | × | × | × | × | — |
| | 1:400 | × | × | × | × | × | — |
| | 1:500 | × | × | × | × | × | — |
| Phenol | 1:125 | — | — | — | | | |
| | 1:130 | — | — | — | — | | |
| | 1:175 | × | — | — | — | — | — |
| | 1:200 | × | × | — | — | — | |
| | 1:250 | × | × | × | × | — | — |
| | 1:300 | × | × | × | × | × | — |

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{200}{150} + \frac{250}{250}}{2} = 1.166$$

From these experiments it appears that formaldehyde has a Phenol Coefficient upon *Bacterium xylinum* of 1.05.

The comparative germicidal efficiency of formaldehyde, as shown in these experiments, is much lower than one would have expected. It is true that we have no data on the specific action of this germicide upon the species of bacteria that we employed as a test organism. Since different species of bacteria vary widely in their resistance to different germicides, we need not necessarily expect our results to agree with those derived from investigations on the action of formaldehyde on other species. The literature on the germicidal efficiency of formaldehyde shows a number of apparent contradictions. Sternberg ([1]) quotes Pottevin, who states that formaldehyde in the proportion of

(1) Text Book of Bacteriology.

1:1000 kills vegetative forms of bacteria in from fifteen minutes to several hours. The same author also quotes Vanderlinden and de Buck, who cite instances where formaldehyde was decidedly inferior to corresponding solutions of phenol. When tested upon *Bacillus coli communis* and *Staphylococcuc pyogenes aureus,* a five per cent solution of formaldehyde failed to destroy them. Ordinarily, however, formaldehyde is believed to be efficient as an antiseptic in the proportions of 1:1000, while the antiseptic strength of phenol is 1:333. There is another factor which probably tended to lower the relative germicidal efficiency of formaldehyde in our experiments. We have already observed that the efficiency of formaldehyde is greatly increased at elevated temperatures. The temperature of 20°C., at which the phenol coefficient was derived, was comparatively low. It is very probable that phenol is relatively more active at this temperature than formaldehyde. Our previous experiments on the influence of temperature upon the efficiency of the various germicides showed that they were not all affected alike by elevations in temperature.

## THE PHENOL COEFFICIENT OF FORMALDEHYDE AS AFFECTED BY THE PRESENCE OF ORGANIC MATTER.

We have already referred to the fact that the efficiency of many germicides is greatly impaired in the presence of organic matter. It is on account of the fact that the germicidal action of phenol is not affected by these conditions that it is accepted as a standard of comparisons in experiments of this kind. In order to determine the phenol coefficient of formaldehyde under conditions that would be more comparable with sugar house conditions, experiments were conducted upon the action of formaldehyde on *Bacterium xylinum* in the presence of cane syrup. Anderson and McClintic ([1]) used a peptone gelatine solution in distilled water in their tests. For our purpose the influence of sugar house products upon the efficiency of germicides is of more practical significance.

## Table XXVI.

SHOWING INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:125 | — | — | — | | | |
| | 1:150 | × | — | — | | | |
| | 1:175 | × | × | — | — | | |
| | 1:200 | × | × | × | — | — | — |
| | 1:250 | × | × | × | × | × | — |
| | 1:300 | × | × | × | × | × | — |
| | 1:400 | × | × | × | × | × | — |
| | 1:500 | × | × | × | × | × | × |
| Phenol | 1:200 | — | — | — | | | |
| | 1:225 | × | × | — | — | | |
| | 1:250 | × | × | — | — | — | — |
| | 1:300 | × | × | × | — | — | — |
| | 1:350 | × | × | × | × | — | — |

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{125}{200} + \frac{200}{350}}{2} = .5985$$

TABLE XXVII.

SHOWING INFLUENCE OF ORGANIC MATTER UPON THE GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

| Germicide | Strength | Time Culture Exposed to Action of Germicide 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
|---|---|---|---|---|---|---|---|
| Formaldehyde | 1:125 | — | — | — | | | |
| | 1:150 | × | — | — | — | — | — |
| | 1:175 | × | — | — | — | — | — |
| | 1:200 | × | × | × | — | — | — |
| | 1:250 | × | × | × | × | × | × |
| | 1:300 | × | × | × | × | × | × |
| | 1:400 | × | × | × | × | × | × |
| Phenol | 1:200 | — | — | — | | | |
| | 1:225 | × | — | — | — | | |
| | 1:250 | × | — | — | — | — | — |
| | 1:300 | × | × | — | — | — | — |
| | 1:350 | × | × | × | × | — | — |

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{125}{250} + \frac{200}{350}}{2} = \frac{.625 + .572}{2} = .5985$$

The method employed in these experiments was as follows: Solutions of formaldehyde and phenol were prepared in such strengths that by the addition of 1 cc of a sterile syrup solution they would represent the same dilutions as employed in the previous experiments. For example, if it were desired to test formaldehyde in the proportion of 1:100 a stock solution of 1:80 was prepared. Four ccs of this solution when diluted by 1 cc of the syrup solution would represent a dilution of 1:100. The method of conducting the experiment was, with this exception, exactly the same as that above described. The syrup solution was first sterilized in an Arnold sterilizer for fifteen minutes on three successive days. It was delivered into the seeding tubes by sterile pipettes, after 4 ccs of the different germicides had first been introduced.

(1) Loc. cit.

## Table XXVIII.

SHOWING INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Formaldehyde | 1:125 | — | — | — | | | |
| | 1:150 | × | × | — | — | | |
| | 1:175 | × | × | — | — | — | — |
| | 1:200 | × | × | × | × | × | × |
| | 1:250 | × | × | × | × | × | × |
| | 1:300 | × | × | × | × | × | × |
| | 1:400 | × | × | × | × | × | × |
| Phenol | 1:200 | — | — | — | | | |
| | 1:225 | × | — | = | — | | |
| | 1:250 | × | × | — | — | — | — |
| | 1:300 | × | × | × | × | — | — |
| | 1:350 | × | × | × | × | × | — |

× Indicates growth.
— Indicates no growth.

Phenol Coefficient $= \frac{\frac{125}{200} + \frac{175}{300}}{2} = 6045$

An average of these three experiments gives formaldehyde a coefficient of .6015, which is considerably lower than the result obtained in the experiments without organic matter. This decrease in its phenol coefficient appears to be due to an increase in the germicidal power of phenol, as well as to a slight decrease in the germicidal efficiency of formaldehyde. In the two series of the experiments the average strengths of formaldehyde and phenol required to prevent growth in a 2½ and 15 minute exposure was as follows:

| Germicide | Without Organic Matter | | With Organic Matter | |
|---|---|---|---|---|
| | 2½′ | 15′ | 2½′ | 15′ |
| Formaldehyde ............ | 1:150 | 1:250 | 1:175 | 1:191 |
| Phenol .................... | 1:158 | 1:233 | 1:200 | 1:333 |

The decrease in the germicidal efficiency of formaldehyde in the presence of cane syrup may be explained by the results of the previous experiments on sugars and fermenting syrup solutions. In these experiments the low efficiency of formaldehyde was attributed to the formation of some combinations between the formaldehyde and the sugars present. The increase in the germicidal action of phenol in the presence of syrup is not so easily explained. That it was not merely due to changes in the composition of the solutions was proven by repeated standardization of the stock solutions. There is a law of germicidal action which may account for this increased efficiency of phenol under these conditions. This law is known as "The Choice of Solvents" ( 1 ). That is to say, the action of a germicide is increased in the proportion in which it is more soluble within the cells of the microorganisms than outside. A germicide has the choice, so to speak, of penetrating the cell or remaining in solution on the outside, and the former course will be followed to the extent in which it offers the lesser resistance. For example, the addition of salt to an aqueous phenol solution increases its germicidal action because it is less soluble in the salt solution. There are cases, however, where the germicidal action depends upon dissociation, in which the above principle does not apply. For example, the addition of sodium chloride decreases the germicidal action of bichloride of mercury because it decreases its dissociation.

It seems probable that the increased efficiency of phenol in the experiments with syrup was due to its lower solubility in that solution.

(1) Bennecke Bau und Leben der Bakterien.

## THE PHENOL COEFFICIENT OF CHLORIDE OF LIME.

The experiments upon the comparative efficiency of phenol and chloride of lime brought out a very surprising fact. It was found that *Bacterium xylinum* is intensely sensitive to chlorine. The stock solutions of chloride of lime were made and standardized by Penot's method ( 1 ), which consists in titrating it with an arsenious solution. At first the dilutions were from 1 : 50 to

(1) Sutton's Volumetric Analysis, p. 174.

1:150, but as these seemed too strong and no growth occurred in any of the transfers made from them the dilutions were increased. After experimenting with dilutions up to 1:1000 without obtaining any positive results, it was thought that there must have been some defect in the method. The test culture was in good condition, as shown by the fact that the growth in the phenol transfer tubes corresponded with those obtained in previous experiments, and also by the normal rate of growth in the control transfers. The two possible explanations of these apparently inconsistent results were as follows: (1) The amount of chlorine carried into the culture tubes from the tube containing the mixture of chloride of lime and test organism might have been sufficient to have prevented the growth of the bacteria, if they had survived the original exposure. (2) The dilution of the culture from .1 cc to 5 cc might have been so great that none of the bacteria were carried over by the platinum loop into the tubes of culture medium. That the first of these theories could not have accounted for the negative results was proven by the fact that the addition of a platinum loopful of the strongest of the chloride of lime solutions to tubes containing sucrose solution which had been inoculated in the usual way, did not prevent growth, nor was the degree of dilution so great that the transfers from the seeding tubes contained none of the bacteria, because transfers of the same amount of culture from a tube that had been similarly diluted with water always resulted in growth.

## Table XXIX.

SHOWING PHENOL COEFFICIENT OF CHLORIDE OF LIME.

| Germicide | Strength | Time Culture Exposed to Action of Germicide | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½′ | 5′ | 7½′ | 10′ | 15′ | 30′ |
| Chloride of Lime | 1:40,000 | — | — | — | | | |
| | 1:42,500 | × | × | — | — | | |
| | 1:45,000 | × | × | × | — | — | — |
| | 1:50,000 | × | × | × | × | × | × |
| | 1:51,000 | × | × | × | × | × | × |
| Phenol | 1:150 | — | — | — | | | |
| | 1:175 | — | — | — | — | | |
| | 1:200 | × | × | — | — | — | — |
| | 1:250 | × | × | × | × | — | — |
| | 1:300 | × | × | × | × | × | — |

× Indicates growth.
— Indicates no growth.
Phenol Coefficient $\dfrac{\dfrac{40,000}{175} + \dfrac{45,000}{250}}{2} = 204$

The results of the above table show that chloride of lime has a very powerful germicidal action upon *Bacterium xylinum.* The phenol coefficients of the other germicides were not determined. It is probable that of all the other germicides used in the previous experiments only the ammonium fluoride would have shown a positive phenol coefficient, and it is likely that it could not have been used in strengths sufficient to prevent growth in an exposure of 2½ minutes. Furthermore, the results obtained from the use of a single species of bacteria is not sufficiently representative of the relative efficiencies of the germicides under sugar factory conditions to warrant a continuance of these experiments.

## Chapter V.

### THE RELATIVE COST OF THE VARIOUS GERMICIDES PER UNIT OF GERMICIDAL EFFICIENCY.

In order to derive an adequate idea of the relative value, for sugar factory use, of the various germicides used in the preceding experiments, it is necessary to take their respective cost into consideration. A germicide which costs one dollar per gallon may be cheaper than another which costs only fifty cents, if the germicidal efficiency of the former is three times as great. It is essential to a fair comparison of the value of the germicides that we determine from their relative cost and their germicidal efficiency their relative cost per unit of effectiveness. The cost of the germicides per pound was obtained from catalogue prices. The price given for formaldehyde is that which is quoted in the catalogue of Perth Amboy Company for barrel lots. In smaller quantities it costs 12 cents per pound. It is probable that many of the other germicides might be obtained at lower prices than those taken as our basis of comparison. These prices are given in the catalogues and apply to small lots rather than purchases in large bulk. The price in each case applies to grades for technical purposes and not to the C. P. quality. The price given for quicklime is for barrel lots.

The cost per unit of efficiency is derived by assuming that of formaldehyde, which is taken as the standard of comparison, to be 1 in every case. Thus we have the formula: ([1])

$$\frac{\text{Cost of formaldehyde per pound}}{\text{Cost per pound of germicide}} = (\text{cost ratio}) \div \frac{\text{Coefficient of formaldehyde}}{\text{Ccefficient of germicide}} \; (= 1)$$

This formula is used in determining the cost per unit of efficiency of disinfectants by the phenol standardization method, but it is just as suitable where formaldehyde is used as the standard, when the proper substitutions are made.

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(1) Hygienic Laboratory Bulletin No. 82.

## Table XXX.

SHOWING RELATIVE COST OF GERMICIDES PER UNIT OF GERMICIDAL EFFICIENCY.

| | | | Experiments on Cane Juice | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Ferment Juice | | Bucket Experiments | | | |
| | Cost per Pound | At Room Temp. | At Temp. for Max. Form. Coefficient | Slightly Fermented | Badly Fermented | Gummy Juice | Badly Fermented Juice | Fermenting Syrup Sol. | Deteriorated Sugar Sol. |
| Formaldehyde | 9¢ | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Chloride of Lime | 6¢ | 1.77 | 1.67 | .537 | .0579 | .257 | .320 | 2.25 | 3.01 |
| Ammonium Fluoride | 60¢ | 55.6 | 29.6 | 4.14 | 18.8 | 61.1 | 3.66 | 61.7 | 41.9 |
| Bisulphite of Soda | 25¢ | 140.0 | 140.0 | 245 | 16.7 | 30.3 | 14.7 | 308 | 174 |
| Bisulphite of Lime | 35¢ | ..... | ..... | 173 | 40.6 | ..... | ..... | 45.2 | 730 |
| Sodium Fluoride | 25¢ | 96.7 | 70.5 | 128 | 150.0 | 127 | 7.44 | 280 | 48.7 |
| Milk of Lime | 1¢ | 1.33 | 0.175 | 0.411 | .125 | .0966 | .197 | 1.45 | 1.39 |

A study of the above table leads to the following conclusions:

(1) At the prices upon which these calculations were based only two of the germicides have as low a cost per unit of efficiency as formaldehyde.

(2) Formaldehyde is superior to any of the germicides for disinfecting syrup tanks or in removing the microorganisms causing deterioration of sugars.

(3) For cleaning tanks containing fermented juices, either chloride of lime or milk of lime can be substituted for formaldehyde at a reduction of cost per unit of efficiency.

(4) In cases where the efficiency of the germicide involves any great penetrating power, as, for example, where gums are to be removed, chloride of lime is a more economical germicide than formaldehyde.

(5) Since the efficiency of all germicides is greater at elevated temperatures, they should always be applied hot in cleaning sugar factories.

(6) The comparatively high germicidal efficiency of milk of lime, combined with its very low cost, and the fact that it is always on hand in sugar factories, renders it particularly suitable as a sugar factory germicide.

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